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Air Pollution Assessment of Nitrobenzene

Mitre Corp, McLean, Va

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Air Pollution Assessment of Nitrobenzene

J. DORIGAN
J. HUSHON

MAY 1976

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16. ABSTRACT Nitrobenzene is a pale yellow oily liquid with a characteristic bitter almond odor and a low volatility. In 1971, 690 million pounds were produced by seven companies, and yearly production increases of 7 to 10 percent are predicted. Bacteria will degrade nitrobenzene in the liquid effluent stream if it is presented to them in a sufficiently diluted state. As shown by the downwind model, the recommended TLV could be reached in 25 hours if no control device were employed. Nitrobenzene can cause chronic toxicity in the industrial environment. There are three routes of uptake: inhalation of vapor, cutaneous absorption of the vapor or liquid, and oral ingestion. There are four sites of physiological reactions to nitrobenzene: blood, nervous system, peripheral metabolism and skin. The immediate toxic effect is methemoglobinemia which is reversible at a constant rate. At higher levels of nitrobenzene accumulates in the cerebellum, affecting the central nervous system. The ingestion of alcohol greatly augments nitrobenzene's toxic effects. Teratogenic and carcinogenic roles for nitrobenzene have been predicted, but there is a need for further testing in this area.

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ABSTRACT

Nitrobenzene is a pale yellow oily liquid with a characteristic bitter almond odor and a low volatility. In 1971, 690 million pounds were produced by seven companies, and yearly production increases of 7 to 10 percent are predicted. Ninety seven percent of all nitrobenzene produced is immediately reduced to aniline, thus eliminating hazards associated with intermediate storage and transport. Principal production losses occur at the reaction vessel and acid concentrator vents and as a contaminant in spent acid (this being the most severe). Bacteria will degrade nitrobenzene in the liquid effluent stream if it is presented to them in a sufficiently diluted state. Present control technology is believed to be adequate to minimize releases. However, as shown by the downwind model, the recommended TLV could be reached in 25 hours if no control device were employed.

Nitrobenzene can cause chronic toxicity in the industrial environment. There are three routes of uptake: inhalation of vapor, cutaneous absorption of the vapor or liquid, and oral ingestion. Liquid absorption presents the greatest danger since, due to its affinity for fats, nitrobenzene penetrates intact skin at a rate of 2 mg/cm^2/hour. The efficiency of nitrobenzene vapor absorption through the lungs is twice that through the skin, but due to its characteristic odor, large exposures are generally avoidable.

There are four sites of physiological reactions to nitrobenzene: blood, nervous system, peripheral metabolism and skin. The immediate

toxic effect is methemoglobinemia which is reversible at a constant rate. At higher levels of nitrobenzene, sulfhemoglobin is irreversibly formed and nitrobenzene accumulates in the cerebellum, affecting the central nervous system. The ingestion of alcohol greatly augments nitrobenzene's toxic effects.

Teratogenic and carcinogenic roles for nitrobenzene have been predicted, but there is a need for further testing in this area.

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I. SUMMARY, AND CONCLUSIONS

Nitrobenzene is a pale yellow, oily liquid at room temperature with a characteristic bitter almond odor. It is only slightly soluble in water (0.1 to 0.2 gm/100 ml), is miscible with most organic solvents, and is an excellent solvent for organic compounds. The volatility of nitrobenzene is low (0.3 mm Hg at 25°C), with a vapor density 4.24 times that of air. Nitrobenzene is relatively unreactive to light and is noncorrosive, but it is readily hydroxylated and, at high temperatures, undergoes reduction to aniline. Reduction of nitrobenzene to aniline accounts for its most important industrial use, consuming 97 percent of the nitrobenzene produced. A minor use is as a solvent for Friedel-Crafts alkylation reactions.

Nitrobenzene is a chemical that evokes chronic toxicity effects in humans. There are three routes for its uptake: inhalation of the vapor, cutaneous absorption of the vapor or liquid, and oral ingestion. Of these, only the first two constitute probable realistic industrial or general population exposure mechanisms.

Due to its affinity for fats, nitrobenzene readily penetrates even intact skin at rates of 2 mg/cm^2/hour and is taken up by blood lipids. Cutaneous absorption from diaper markings resulted in reversible cyanosis and labored breathing in infants, and 0.7 gm/kg administered on the skin proved fatal in two days to rabbits.

The efficiency of nitrobenzene vapor absorption in the lungs is twice that through the skin. A man exposed to the current OSHA (Occupational Safety and Health Administration) standard for nitrobenzene of 1 ppm (eight-hour time weighted average) would be expected to absorb aproximately 24 mg by inhalation and 9 mg cutaneously in an eight-hour period. Thus, a maximum eight-hour uptake of 33 mg is possible at the TLV (threshold limit value), which is less than the "reasonably safe" dose of 35 mg/day. Dosages exceeding 35 mg/day have been reported for nitrobenzene factory workers in England (70 mg/day maximum) and in a dye stuff factory (80 mg/day maximum). However, most factory workers absorb less than 35 mg/day.

Instances of oral ingestion are generally due to suicide attempts. Some accidental intake was also reported. The amount of nitrobenzene ingested and the physiological responses were extremely varied, ranging from death after intake of 0.4 ml to recovery after ingestion of 333 ml. Probably the major factor affecting the level of toxicity in man and animals is the presence of alcohol.

Physiologically, there are four reactions to nitrobenzene exposure: in the blood, nervous system, in the peripheral metabolism, and skin disorders. The immediate toxic effect is methemoglobinemia, evidenced by such symptoms of oxygen insufficiency as labored breathing. Methemoglobin reverses to hemoglobin at a constant rate, so that low chronic or isolated, higher exposures to nitrobenzene may be tolerated. Higher levels of nitrobenzene cause more lasting and severe anemia, not only by forming methemoglobin at rates

exceeding normal reversal but also by producing sulfhemoglobin, irreversible to hemoglobin. While nitrobenzene is directly responsible for a small amount of methemoglobin formation, the greater amount of this formation appears to be caused by metabolites of nitrobenzene such as aniline and aminophenol.

At higher doses and longer exposures, nitrobenzene appears to accumulate in the cerebellum, affecting the central nervous system, and in body organs, such as the liver. Accumulation is due to a faster rate of nitrobenzene uptake than metabolism and excretion. Lethal effects apparently result from nerve (brain) damage, affecting respiration, while the chronic, reversible effects are due to blood changes. Symptoms of acute poisoning in man and animals include uncontrolled eye movement, coma and convulsion. Acute toxicity also causes liver damage and bone marrow changes. Skin dermatitis is a rare result. Absorbed nitrobenzene usually produces effects internally; however, skin inflammation from chronic nitrobenzene vapor exposure and from contact with inks containing nitrobenzene has been reported.

Rats injected subcutaneously with 125 mg/kg nitrobenzene had both delayed and abnormal embryo development. Pregnant women exposed to factory vapors were found, in general, to have decreased circulation to their fetuses, but no follow-up studies on the newborns regarding human teratogenicity were reported. However, the teratogenic effects found in rats suggest that pregnant women should avoid nitrobenzene exposure.

Carcinogenicity of nitrobenzene is purely theoretical, based on the cellular proliferation response to other intoxicating compounds. However, nitrobenzene was found to sensitize cells to x-rays and decrease cellular proliferation in cultures of human and hamster cells.

Metabolic products of nitrobenzene appear similar in humans and animals. Major excretory compounds are aminophenol and nitrophenol. In rabbits, approximately 50 percent of the nitrobenzene is accumulated in tissues within two days after 0.25 ml intubation. In four to five days, 70 percent is excreted through urine, air and feces, and in eight days, only eight percent of the dose remained in rabbit tissues exclusively in the form of nitrobenzene metabolites.

Bacterial degradation of nitrobenzene was observed in synthetic sewage and in a waste water purifier inoculated with Azobacter agilis. Cultures, cells and extracts of Nocardia erythropolis and Pseudomonas fluorescens apparently reduced the nitro group to the amino level via the nitroso and hydroxyamino stages. These degradations are important, since nitrobenzene may be released in the waste waters from industrial plants producing nitrobenzene, aniline and dyestuffs. Nitrobenzene was also found to be toxic to bacteria. Benzene-acclimated sludge had oxygen uptake halved by the addition of nitrobenzene, and sulfate-reducing bacteria had growth delayed or prevented by 2 to 20 ppm nitrobenzene. Another culture, Azobacter vinelandii, did not utilize nitrobenzene, presumably because reduction to NH_3 was impossible.

There are seven companies producing nitrobenzene in the United States. Primary production locations are sited on the Gulf Coast, and in Philadelphia, New Jersey and West Virginia. Total production capacity for nitrobenzene is 1,065 million pounds per year, but only 690 million pounds were produced in 1971. Yearly increases of 7 to 10 percent are predicted as the demand for aniline increases. Nitrobenzene is usually manufactured by direct nitration of benzene using mixed sulfuric and nitric acids. Newer continuous methods employ vapor phase nitration in tubular reactors.

Principal production losses occur in the reaction vessel, acid concentrator vents, and as a contaminant in the spent acid. An estimated 8.3 million pounds of nitrobenzene are released yearly into the environment when sulfuric acid reconcentration is practiced. Activated charcoal absorbers or a carbon dioxide blanket are employed to prevent nitrobenzene escaping into the atmosphere. These measures appear to be sufficient controls. Atmospheric concentrations of nitrobenzene have not been monitored by industry, but levels are assumed to be far below inner plant levels which, in turn, remain below the TLV. Work area vents should be located in hazardous areas and on floors in addition to wall or ceiling vents. The specific gravity of the vapor (4.2) indicates a tendency for the gas to accumulate at floor level.

Most nitrobenzene loss occurs during acid washes and is carried in the effluent waste water. This residual constitutes the greatest pollution threat. Seeding of water treatment facilities with known

nitrobenzene degrading bacteria, such as Azobacter agilis, could significantly reduce this residual. Other effective control methods for the acid effluent include recycling water from the condensor and cooling system, neutralization of the water and denitrification. Acute nitrobenzene toxicity to aquatic microorganisms occurs at approximately 30 mg/liter. At 0.03 mg/liter, biochemical oxidation and nitrobenzene degradation are inhibited, which provides sufficient warning before any acute toxic effects occur.* Effluent wastewater requires continuous monitoring to keep nitrobenzene at levels low enough to allow biodegradation.

Nitrobenzene is almost entirely used as a synthetic intermediate, so there is no serious liquid disposal problem. However, should nitrobenzene have to be dumped, the receiving area should be lined to prevent soil percolation through the soil. Chemical inactivity of nitrobenzene makes it a persistant species in the environment. Furthermore, its resistance to degradation by many microorganisms and its toxicity to others, provides the basis for the classification of nitrobenzene as hazardous.

The extremely variable response to nitrobenzene exposure necessitates close observation of individuals, so that adverse chronic effects may be remedied before latent, acute effects occur. Precautions, such as daily air monitoring, are advised in areas of high risk or suspected contamination.

*Some toxic species, if diluted sufficiently, can be assimilated and degraded by bacteria. However, should concentrations rise above the assimilation level, toxicity reduces the bacterial population.

Aniline production is conducted as a continuous process with a nitrobenzene feedstock. Ninety seven percent of all nitrobenzene is consumed directly in this fashion, thereby eliminating any storage and transport emission problems. Since nitrobenzene is usually kept in a closed system with minimal release to the ambient air, it appears that at most, a minor air contamination problem may be expected. A concentration of 40 mg/25 hours, the resultant emissions calculated from a hypothetical plant sampled 500 meters downwind from the facility, is approximately equal to the maximum permissible dosage set by the American Conference of Governmental Hygienists and emphasizes the necessity for air emission controls. Some evidence exists of the atmospheric formation of nitrobenzene by the photochemical reaction of benzene with nitrogen oxide.

II. AIR POLLUTION ASSESSMENT REPORT

A. PHYSICAL AND CHEMICAL PROPERTIES

At room temperature nitrobenzene is a pale yellow, oily liquid with low volatility and a characteristic bitter almond odor. The liquid is only slightly soluble in water but miscible with most organic solvents. There is a medium fire hazard associated with nitrobenzene, as exposure to approximately 90°C is required before ignition is possible (1 to 12). Physical properties are listed in Table I.

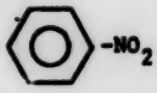
Nitrobenzene is the simplest of the aromatic nitro compounds. It is also referred to as oil or essence of mirbane (myrbane), nitrobenzol, C.I. Solvent Black 5, artificial oil of bitter almonds, mononitrobenzene, and the trade name Nigrosine Spirit Soluble B (5). It is only slightly volatile at ordinary room temperature (0.284 mm Hg) (1, 19) and is not considered to be a volatile organic compound by the Code of Federal Regulations* (77.6 mm Hg at storage conditions).

Nitrobenzene may persist in the environment unless it is reduced by microorganisms (8), although it is likely to settle out of the air as a result of its greater density. The compound is not readily photolyzed nor is it corrosive (7). It is relatively unreactive with organic peroxides and ozone, the respective rates of oxidation having half-lives of >2,000 years and 11 years (18). Nitrobenzene is very

*Title 40. Protection of the Environment. Part 52.

TABLE I
PHYSICAL PROPERTIES OF NITROBENZENE

Molecular formula: $C_6H_5NO_2$

Structural formula: 

Molecular weight: 123.11

Freezing point: 5.6 - 5.7°C

Solubility: Water	gm/100 ml	°C
	0.1-0.239*	20.
	0.16	24.4
	0.8	80
	1.0	100

Solvents: ethanol, diethyl ether, acetone, benzene, lipids

Vapor pressure at:		
	199.5°C	600 mmHg
	192.5°C	500 mmHg
	184.5°C	400 mmHg
	174.5°C	300 mmHg
	160.5°C	200 mmHg
	139.9°C	100 mmHg
	120.2°C	50 mmHg
	84.9°C	10 mmHg
	53.1°C	1 mmHg
	25. °C	0.284 mmHg

*Differences in solubility values cited were more than two-fold, but the values for water solubility remain low.

TABLE I (Continued)
PHYSICAL PROPERTIES OF NITROBENZENE

Vapor density (air = 1.0):	4.24
Log partition coefficients:	Hexane/water - 3.18 at 24.4°C Octanol/water - 1.85 - 1.80 at 24.4°C
Diffusivity:	1.8×10^{-5} cm^2 sec^{-1} in benzene
Density:	1.2229 gm/ml at 0°C* 1.2193 gm/ml at 4°C* 1.3440 gm/ml at 1.5°C 1.2125 gm/ml at 10°C 1.205 gm/ml at 15°C 1.205 gm/ml at 18°C 1.2037 gm/ml at 20°C 1.1986 gm/ml at 25°C
Flash point (closed cup):	87.8°C
Autoignition temperature:	482.2°C
Flammable limit in air (explosive limit):	1.8% by volume at 93.5°C
Fire hazard (N.F.P.A.):	medium (must be moderately heated or exposed to relatively high ambient temperature before ignition can occur). Burns with a sooty, yellowish flame. Fires extinguished by water, foam, CO_2 or dry chemicals.
Heat of combustion:	739.2 k cal/mole at 760 mmHg at 20°C

*Supercooled liquid.

TABLE I (Concluded)
PHYSICAL PROPERTIES OF NITROBENZENE

Latent heat of fusion: 22.4 cal/gm
Latent heat of vaporization: 79.08 cal/gm
Dipole moment: 3.97 Debyes in benzene at 22-25°C
Viscosity: 3.083 cp * at 0.3°C **1.682 cp at 30°C** **0.704 cp at 100.5°C**
Characteristic oil of bitter almond odor **detection level - 10^{-4} m moles/liter (10^{-3} -10^{-5})** **recognition level - 10^{-1} m moles/liter (10^{-1} -10^{-2})** **odor threshold in air by volume - 0.5 ppm**

*cp = centipoise

SOURCES: 1 - 17.

reactive with the hydroxyl ion (half-life of three days) (18) and reactive with reducing agents and caustic soda at high temperatures (7).

The most important industrial reaction of nitrobenzene is reduction to aniline (1). It is sometimes used as a convenient, mild oxidizing agent for the oxidation of isocugenol to vanillin, in the fuchsin fusion reaction, and in the synthesis of quinoline (1). However, it can also be of sufficient strength to oxidize sodium methoxide to sodium formate.

Nitrobenzene is an excellent solvent for organic compounds and is employed as a crystallizing solvent for substances that are practically insoluble in more common organic solvents (9). It has a disadvantage during crystal drying because of its low volatility and mild oxidizing action at elevated drying temperatures.

Nitrobenzene is further nitrated using fuming nitric acid to form dinitrobenzene. It reacts with fuming sulfuric acid to give nitrobenzene-sulfonic acid. Catalyzed chlorination produces nitrochlorobenzene (9). When warmed with powdered potassium hydroxide, the chemical is converted in part to nitrophenol azoxybenzene (9).

Nitrobenzene has one double and one semipolar bond, causing the nitro group not only to be unsaturated but to have a positively charged atom in a key position next to the benzene ring (meta orienting). The nitro group can be reduced under a variety of conditions to yield arylamine, arylhydroxylamine, azoxy-, azo-, or hydrazo compounds or various rearrangement products (1).

In electrophilic conditions, the nitro group exhibits its normal meta-directing character. The resonance effect in nitrobenzene establishes positive centers at the ortho and para positions, rendering those sites particularly inaccessible to electrophilic agents. The meta carbon atoms, while deactivated along with the rest, offer better sites for attachment than the ortho and para positions (9). It is this meta directing property that enables nitrobenzene to be used as a solvent for the Friedel-Crafts reaction (20).

B. EFFECTS

The ancient Greeks were apparently familiar with a substance with a similar physiological action to that of nitrobenzene (6):

> It is said that Thrasyas, the father of botany, was so skilled in the preparation of drugs, that he knew how to compound a poison which would...kill by a lingering illness. Theophrastus speaks of this poison, and says its force could be so modified as to occasion death in two, three, or six months, or even at the end of a year or two years; and the writings of Plutarch, Tacitus, Quintilian and Livy are full of instances of what seem to be the same kind of slow and occult poisoning.

However, nitrobenzene was unknown to modern chemistry until its discovery in 1834 by Mitscherlich (6). The poisoning effects of nitrobenzene were noted as early as 1856 (6). Since that time, numerous accidental and intentional fatalities have been reported. Experiments with humans and animals have been conducted for the purpose of studying the toxic action of nitrobenzene.

1. Human

Exposure and Uptake. There are three routes for nitrobenzene uptake: vapor inhalation, cutaneous absorption (liquid or vapor), and ingestion (liquid). Of these routes, only the first two constitute serious industrial or general population exposure mechanisms.

Cutaneous absorption is the greatest hazard in industry and appears to cause the most marked toxic effects. Absorption of nitrobenzene was thought by early investigators to be a slow process. However, its high fat-solubility permits it to penetrate through the gastrointestinal tract, lungs and skin. Absorption rates as high as 2 mg/cm^2/hour for the material have been reported (21).

Nitrobenzene poisonings caused by cutaneous absorption of dyes containing nitrobenzene have been reported (5). Usually, the poisonings were not severe and recovery ensued within 24 hours. Infants wrapped in diapers stamped with a nitrobenzene dye showed signs of poisoning (cyanosis*, shallow breathing, vomiting) that was reversed within 24 hours of diaper removal (5). However, a man wearing a pair of shoes stained with liquid shoe-black containing nitrobenzene died a few hours after drinking beer (6). The synergistic effect of alcohol with the absorbed chemical undoubtedly contributed to the lethality. These cases are interesting because a small quantity of nitrobenzene was able to produce severe or fatal effects. Absorption of nitrobenzene during use as a parasiticide has given rise to a range of reported poisonings (5).

The absorbed quantities of nitrobenzene vapor increase as the ambient concentration increases (21). Normal work clothes reduce this absorption 20 to 30 percent. Cutaneous absorption rates and absorbed quantities for dressed and naked men at different nitrobenzene concentrations are given in Table II. Increased humidity increases the rate of nitrobenzene absorption when the temperature remains constant. Absorbed doses and absorption rates of nitrobenzene vapors are given for different humidity and temperature conditions in Table III. An average absorption dose of 13 mg of nitrobenzene was received after six hours cutaneous exposure to nitrobenzene vapor levels of 10 mg/liter (21). Dosage increased approximately 2 mg with either

*Bluish discoloration of skin and mucous membranes.

TABLE II

ABSORPTION OF NITROBENZENE VAPOR THROUGH THE SKIN AT DIFFERENT CONCENTRATIONS

Series	Number of Experiments	Nitrobenzene Concentration (μg/l)	Absorbed Doses of Nitrobenzene (mg)	Absorption rate per unit of concentration (mg/hr: μg/l)	Notes
1	3	5	11, 7, 4 (mean 7)	0.23	dressed
2	3	10	16, 14, 8 (mean 13)	0.22	dressed
3	3	10	19, 16, 10 (mean 15)	0.25	naked
4	3	30	80, 37, 46 (mean 54)	0.30	naked

Temperature 25°C; humidity 35%; exposure time 6 hours.

Source: Piotrowski, J. "Further investigations on the evaluation of exposure to nitrobenzene." benzene. British Journal of Industrial Medicine 24(1):60-65, 1967.

TABLE III

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE ABSORPTION OF NITROBENZENE VAPOR THROUGH THE SKIN

Series	Number of Experiments	Temperature (0°C)	Humidity (%)	Absorbed doses of Nitrobenzene (mg)	Absorption rate per unit concentration (mg/hr: µg/l)
3	3	25	35	19, 16, 10 (mean 15)	0.25
5	3	30	25	13, 16, 10 (mean 13)	0.22
6	4	25	67	18, 18, 34, 24 (mean 23)	0.38

Air concentration 10 µg/l; persons naked; exposure time 6 hours

Source: Piotrowski, J. "Further investigations on the evaluation of exposure to benzene. British Journal of Industrial Medicine 24(1):60-65, 1967.

a 10 percent humidity increase or removal of clothing. Vapor concentration at the threshold limit value (TLV) of 5 mg/m^3 resulted in an average absorption dose of 7 mg after six hours cutaneous exposure (21).

Clinical records of nitrobenzene poisoning by vapor inhalation include those of a man who, after working for approximately three weeks in a nitrobenzene factory filling containers with the chemical, suffered permanent cerebellar* effects (6). Women employed by a paint firm and exposed for approximately 17 months during nitrobenzene use as a solvent gradually recovered from the toxic effects in two weeks (22). Serious poisonings have also resulted from inhalation of nitrobenzene used as a scent in inexpensive perfume and soap (5, 79).

Nitrobenzene vapor is rapidly absorbed through the lungs with 80 percent retention. Lung absorption rates are twice the cutaneous values (21). Nitrobenzene retention in the lungs averaged 87 percent the first hour and 73 percent in the sixth hour of exposure to 6 to 30 g/liter. The lower retention is probably due to blood saturation and the low conversion rate of the compound (23). In Poland, the maximum acceptable concentration (M.A.C.) is 5 mg/liter or 25 mg/day (21). At this level, the average worker in a nitrobenzene factory in Poland could cutaneously absorb 7 mg of the material, assuming he is exposed a full workday of 6 hours. In addition, he could absorb about 18

*Pertaining to that part of the brain concerned with coordination of movements

mg/day via the lungs (assuming a lung ventilation of 4 to 5 m^3 and 80 percent retention). Thus, the uptake would be 25 mg/day, or the M.A.C. Although cutaneous absorption of vapor is much lower than lung absorption, it contributes approximately one-third to the total absorbed concentration. In calculating total uptake, the activity of the worker must also be considered. A man sitting down has reduced lung ventilation and may be exposed to higher concentrations without absorbing dangerous levels of nitrobenzene (21).

Oral ingestion of nitrobenzene has been reported as a result of suicide attempts, accidental ingestion, uses as an abortive agent, and as an artificial almond flavoring (5,6,24). During the late nineteenth century, nitrobenzene gained a reputation as an abortive agent. In most instances, the desired effect was not accomplished, as the nitrobenzene was equally toxic to both mother and fetus. Of 24 cases examined, twelve proved fatal to the mother (5).

As late as 1949, nitrobenzene was used as an artificial almond flavor. When five nurselings in a hospital suddenly became cyanotic, it was found that their mothers had eaten cake which was artifically almond-flavored with nitrobenzene and thus had transmitted the compound to the child via their milk. Adverse effects disappeared within 24 hours after cessation of exposure (24). At present, nitrobenzene is no longer on the FDA approved list of food additives.

Metabolism. Nitrobenzene, detected in human urine, was excreted both unchanged and partly metabolized (5, 22, 23). It is metabolized slowly, with a maximum rate independent of dose in the later stages of

acute intoxication from either chronic or acute exposures (21, 25). Thus, nitrobenzene accumulation in high-lipid tissues may occur during chronic exposures at concentrations above the rate of catabolism.*

The metabolic conversion rate for nitrobenzene was determined by oral experiments involving one dose of either 5 mg p-nitrophenol or 30 mg nitrobenzene (21). The p-nitrophenol was rapidly excreted in the urine, while the nitrobenzene was slowly excreted as the metabolite p-nitrophenol. Myslak et al. (25) found delayed periods for excretion of the nitrobenzene metabolites, p-aminophenol and p-nitrophenol, to be 60 hours after a 50 ml dose of nitrobenzene. Analysis of the subject's urine indicated p-aminophenol was present on the first day with maximum excretion (198 mg/day) on the second day, and p-nitrophenol present on the second day, with maximum excretion of that metabolite (512 mg/day) also on the following day (25). Over a period of 22 days, totals of 712 mg of p-aminophenol and 1780 mg of p-nitrophenol were excreted.

Hamblin (12) suggests that detoxification of nitrobenzene is accomplished first with rearrangement to o- and p-aminophenols or by reduction to aniline. Subsequently, the aniline is excreted as an acetyl derivative or hydroxylated and excreted as an aminophenol. The aminophenols, being more water soluble than nitrobenzene, would be more readily excreted through the kidneys. Von Oettinger (5) suggests that nitrobenzene is reduced to aniline via nitrosobenzene

*Breaking down by living organisms of more complex substances into simpler ones.

or to p-aminophenol via phenylhydroxylamine and subsequently excreted as the aminophenol alone or conjugated with glucuronic acid.

Salmowa et al. (23) proposed quantification of ambient levels of nitrobenzene exposure based upon its relationship to p-nitrophenol excreted in the urine of exposed individuals. It was found that an experimentally determined range in urine collected two hours after exposure can be used to determine the nitrobenzene exposure levels (see Figure 1). If p-nitrophenol values are very high and exceed the experimentally determined range, nitrobenzene exposure can be calculated as follows (23):

$$x = y/2.6$$

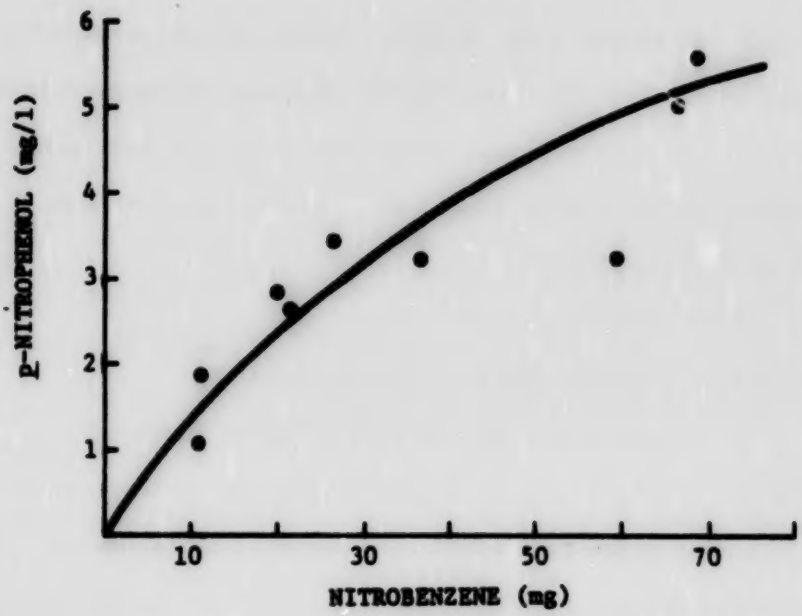
where x = absorbed dose of nitrobenzene (mg)

y = excretion rate of p-nitrophenol (mg/hour)

The application of these determinations is limited to short-term exposures, because there is accumulation during long exposures. The maximum possible exposure can be ascertained even in chronic exposures, but the dosage may be greatly exaggerated. Piotrowski (21) would use the same method of calculation but suggests sampling on the last three days of the work week to estimate the daily dose. This would coincide with the time of maximum p-nitrophenol excretion that his experimentation revealed (third to fourth day after exposure).

Physiological Reactions. The onset of nitrobenzene poisoning is insidious. The exposed subject may feel well, even euphoric on occasion. Appearance of symptoms depends upon the nature and

FIGURE 1
CONCENTRATION OF p-NITROPHENOL IN URINE COLLECTED 2-3 HOURS AFTER END OF EXPOSURE TO NITROBENZENE



Source: Salmowa, J., J. Piotrowski and Y. Neuhorn. "Evaluation of of exposure to nitrobenzene. Absorption of nitrobenzene vapour through lungs and excretion of p-nitrophenol in urine." British Journal of Industrial Medicine 20(1):41-49, 1963.

intensity of exposure as well as the physiological state of the subject. There may be a considerable time delay before outward symptoms are manifested. However, a marked formation of methemoglobin* occurs rapidly and may be detected by blood analysis before physiological effects are observed. The latent period between actual poisoning and the appearance of the first signs may vary from one to more than four hours (5). It appears that metabolic changes in the absorbed nitrobenzene and slowed absorption/storage in the body fat produce the delayed reaction.

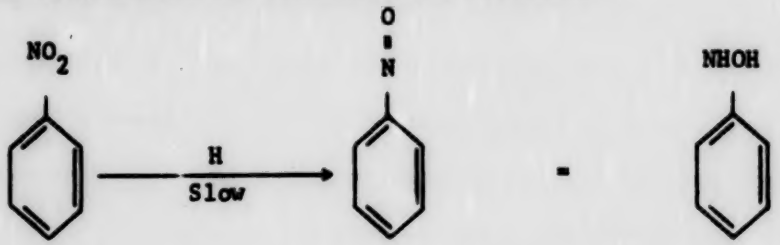
There are four major physiological reactions to nitrobenzene:

- o Blood. Hemoglobin** is converted to methemoglobin and eliminated from the oxygen transport cycle.
- o Nervous system. Headache, nausea, and in large doses, coma and cerebellar effects are observed.
- o Peripheral metabolism. Metabolic processes degenerate rapidly due to excessive consumption of body substances and high body temperature, exemplified by liver damage.
- o Skin. Irritation and sensitization of the skin with eczema*** may occur.

Methemoglobin formation occurs by oxidation of hemoglobin. Hemoglobin transports oxygen in a readily dissociable form and is necessary for life (26). Methemoglobin binds oxygen so that it is unavailable for oxygen exchange. The presence of methemoglobin further renders the remaining hemoglobin less capable of oxygen

*a form of hemoglobin with decreased oxygen carrying capacity
**oxygen-carrying pigment
***inflammatory disease of the skin

dissociation (12). There is some debate whether it is nitrobenzene *per se* that produces methemoglobin or an intermediate metabolite. Hamblin (12) suggests it is a derivative (formed by enzyme-catalyzed reduction of the nitro group to known hemoglobin-complex formers) that is responsible for methemoglobin formation:



Nitrobenzene Nitrosobenzene Phenylhydroxylamine

In vitro experiments showed some direct methemoglobin formation by nitrobenzene (5, 27). However, the major amount is converted by metabolites, such as nitrosobenzene (12) and *p*-aminophenol (6). Small amounts of methemoglobin are normal, differing as to level and reduction rate in various species (12). In all species, including humans, an ultimate equilibrium between methemoglobin and hemoglobin is reached. Methemoglobin concentration does not increase any further even with dosage increases after a certain point (12). Determinations subsequent to a suicidal ingestion of 50 ml of nitrobenzene indicated that 82 percent is the maximum methemoglobin formation to occur in humans (25).

Clinical reports of nitrobenzene poisonings indicate no adverse effects are felt by patients with 40 percent methemoglobin. Dizziness,

dyspnea* upon exertion, and an increased pulse rate occur when methemoglobin concentration is over 40 percent, with increased respiration appended at a methemoglobin concentration of approximately 70 percent (12). The concensus seems to be that methemoglobinemia is responsible for the reversible effects of nitrobenzene poisoning, and that fatalities probably result from other causes (nerve damage).

The general effect on the blood from nitrobezene exposure appears to be methemoglobin formation within the red cell, hemolysis of red blood cells (erythrocytes), and concurrent regeneration of red blood cells (reticulocytes) (see Figure 2) (5). In some instances, increased hemoglobin content was observed (5, 6). While there are no consistent changes in the white blood cells (leucocytes), their numbers increased in acute (but not chronic) nitrobenzene poisoning (5). A number of changes were reported in the bone marrow of workers using nitrobenzene solvents in a glass and procelain factory (28). Hyperplasia** of the lymphoid*** reticular cells predominated, possibly occurring as an early phase of more severe bone marrow lesions. Bone marrow damage was also observed by Myslak et al. (25).

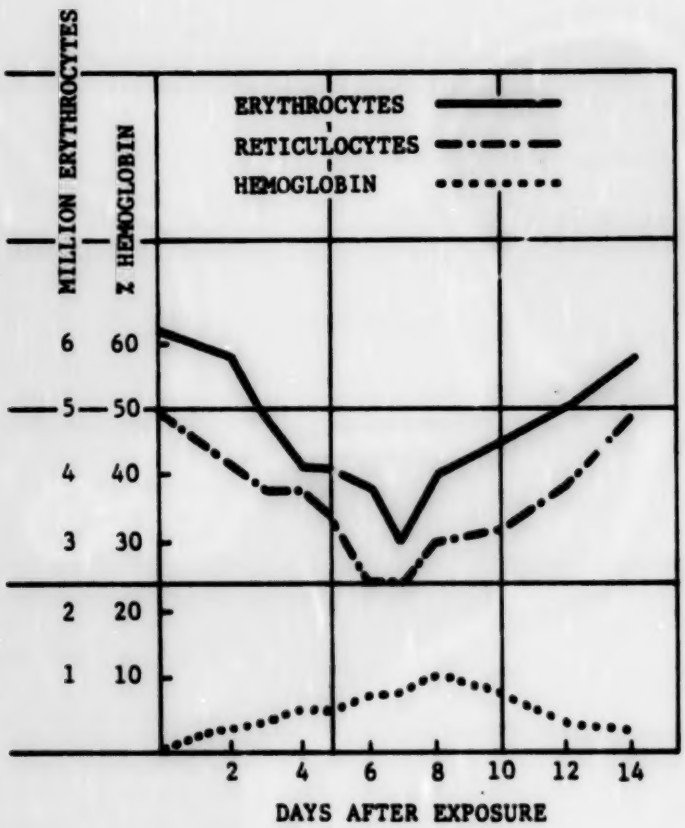
Along with methemoglobin, sulfhemoglobin may be present in the blood of nitrobenzene patients. Interaction of unstable sulfhydryl compounds with methemoglobin in the blood, or by simultaneous exposure

*Labored breathing.

**Abnormal increase in cell number in a tissue.

***Fluid containing white blood cells.

FIGURE 2
DAYS AFTER EXPOSURE NITROBENZENE ACTION ON RED BLOOD CELLS



Source: Von Oettinger, W. F. "The aromatic amino and nitro compounds, their toxicity and potential dangers. A review of the literature." Public Health Bulletin 271. U.S. Public Health Service, Washington, D.C., 1941.

to hydrogen sulfide, results in the formation of sulfhemoglobin (sulfmethemoglobin) (5). This form of methemoglobin is irreversible to hemoglobin (29).

Effects of nitrobenzene exposure depend to some extent on the immediate blood chemistry (6). Thus, the size of a lethal dose and the symptoms of poisoning are extremely variable at different periods of time in the same individual, for the blood chemistry is never absolutely constant. Nitrobenzene is readily absorbed from the blood by liquid body-fats. However, as the equilibrium shifts, nitrobenzene is subsequently released back to the blood and transported to tissues, such as brain and nerves, that have a lesser affinity for nitrobenzene than does body fat (6). At low exposure levels, nitrobenzene may be present in such low concentrations in the blood as to be eliminated from the body without ever reaching sufficient amounts to cause symptoms of poisoning. At higher exposures, uptake will exceed catabolism and excretion, and nitrobenzene will accumulate in sensitive tissues causing toxic effects. Ultimately, it appears that conditions which favor or disfavor a concentration of nitrobenzene in the vicinity of nerve cells will determine the lethal dose (6).

The initial effects of nitrobenzene on the nervous system include headache of varying intensity, fatigue and dizziness. There is progressive depression of the central nervous system resulting in unconsciousness and coma (5). Visual disturbances may be characterized by double vision or nystagmus.* Severe dyspnea may occur,

*Involuntary, rapid movement of the eyeball.

decreasing in patients who suffer from strong depression of the central nervous system (5). Severe cases of nitrobenzene poisoning may end with mental debility. The loss of perception of time and space is the principal feature, indications of cerebellar lesions (5, 6). Nitrobenzene affects the central nervous system by anoxemia*, similar to carbon monoxide poisoning, and the effects depend upon the intensity and duration of exposure (5).

The latent period before brain damage is probably due to absorption of nitrobenzene from the blood by the lipids of the cerebellar or other brain cells until a sufficient concentration is reached to produce the onset of motor symptoms. Possibly the condition of the brain lipids is involved in hastening or retarding absorption of nitrobenzene. Thus, a patient's brain cells may continue to absorb nitrobenzene over a long period of time as it is released from storage in body fats to the blood, necessitating continued observation of patients after apparent recovery.

Nitrobenzene was found to produce central lesions in the nystagmogenic zone in the brain, where peripheral stimuli are transformed into oculomotor** impulses (30). This action was confirmed by the appearance of nystagmus. Cerebellar lesions were also indicated in a man with Korsakoff's psychosis*** who had worked filling nitrobenzene containers (6).

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*Reduction of oxygen content of the blood.

**Eye movement.

***Disturbance of orientation, susceptibility to external stimulation and suggestion, falsification of memory, and hallucinations.

Most deaths appear to be due to nerve damage which results in a cessation of respiration (5). Degeneration of nerves has also been suggested as the cause of hyperalgesia* of the skin, especially in the extremities (22, 29). Other indications of nerve or brain damage include: incoordination, hesitating speech, drowsiness, numbness, convulsions, and dilation or contraction of the pupils (6).

The effects of nitrobenzene on organs other than the brain have been reported from autopsies and clinical records. In the former, a strong almond odor was apparent from all the organs and the body cavity, whereas in the latter, the odor was evident in the urine and breath of patients (5, 25, 31, 32).

The anemiagenic action of nitrobenzene is reflected in enlarged spleens and livers, and jaundice (5, 22, 25). The liver may show degenerative and necrotic areas (5) and the characteristics of cirrhosis (33), with fatal yellow atrophy of the liver and pernicious anemia** as late effects of severe nitrobenzene poisoning (5).

A burning throat and mouth after oral ingestion (29), or congestion of the pharynx*** after inhalation (5) followed by poor appetite (22), stomachache, vomiting (5,6), and diarrhea with bloody stools (5) are indications of nitrobenzene poisoning. Urine may contain pigments (bilirubin, hydrobilirubin) and other constituents of the bile

*Sensitive to pain.

**Severe reduction number of red blood cells.

***The tube that connects the mouth with the stomach.

(urubilin) (5,6,27,31). Hematoporphyrin* is also found in the urine, and may be indicative of nitrobenzene exposure in the absence of other symptoms (5). Most abdominal organs are filled with blood (5), with damage to the enlarged heart as well (5,25,31). Lungs are edemic, and the lung cavity fills with blood (5,31).

Menstrual disturbances occurred after chronic exposure to nitrobenzene (5). Body temperatures of patients were elevated to 37°C(100.4°F) (5, 31). Later complications from nitrobenzene poisoning were attributed to visceral storage of the chemical, especially in fat tissue, and subsequent release (29).

Changes in the tissues of the chorion** and placenta*** were observed in pregnant women who worked in the production of a rubber catalyst that uses nitrobenzene (34). Decreased circulation in blood vessels and necrotic changes in tissues were found. No mention was made of effects on fetal development or death, as was found for rats exposed to nitrobenzene (53,54).

Contact dermatitis**** was felt to be rather rare by Calnan and Connor (35). They found that reported nitrobenzene afflictions generally were due to other causes. However, they documented one patient allergic to carbon paper containing nitrobenzene. Von

*A product of the decomposition of hemoglobin.

**Outermost envelope of the fertilized egg.

***Organ which establishes communication between mother and child by means of the unbilical cord.

****Inflammation of the skin.

Oettinger (5) reports skin irritation from long-term exposures to chronic nitrobenzene vapor levels.

Alcohol may have a synergistic effect with nitrobenzene, as toxic symptoms become more pronounced after drinking alcoholic beverages. One nitrobenzene factory worker evidenced some symptoms of poisoning, such as headache, stomachache and vomiting during the work week. By Sunday, he felt much better. Subsequently, he drank two glasses of beer, and collapsed unconscious (6). The ingestion of alcohol (or chronic alcoholism) has been found to lower the lethal or toxic dose of nitrobenzene (5). Since ingestion of fatty oils showed similar results, it appears the effects are due to the increased solubility of nitrobenzene in these materials.

Carcinogenicity of nitrogenzene is suspected only. Passon (36) draws a parallel between visible results of intoxication such as rheumatoid arthritis* and retrobular neuritis** and the appearance of the carcinoma. The same chemicals (e.g., nitrobenzene) that produce cellular intoxication at high doses may, at low doses, lead to uncontrolled cellular proliferation and eventual malignant degeneration. This development is highly dependent on constitutional factors and the intervention of the autonomic nervous system.*** Nitrobenzene has been found to sensitize hypoxic**** cells to x-rays

*Inflammation of the joint.

**Inflammation of the nerve.

***Nerves that control involuntary functions.

****Oxygen-poor.

(37, 38). A direct correlation between cytotoxicity* and drug electron affinity was reported in human cell cultures.

Symptoms of nitrobenzene poisoning vary considerably in character and time of appearance. They are dependant upon the interaction between exposure level and physiological conditions. The following groups of symptoms are given without reference to specific time intervals (5, 6, 19, 22, 25, 29, 31, 33, 34, 39):

Chronic exposure:

- fatigue
- cyanosis
- dizziness
- headache
- methemoglobinemia (low level)
- stomachache
- vomiting, nausea
- almond odor to urine
- dyspnea
- chocolate brown blood (high level methemoglobin)
- painful liver
- hypersensitive skin

Long-term chronic and/or acute exposures:

- irregular pulse
- incoordination
- low blood pressure
- prostration
- elevated temperature
- nystagmus
- pupils react sluggishly, dilated or contracted
- unconsciousness
- confusion
- convulsions
- coma
- mental debility

*Toxicity to cells.

The symptoms listed may not appear in the order indicated above, or appear at all. But generally, fatigue and cyanosis are first observed, and convulsions and coma occur only in critical cases.

Prophylactic measures are usually employed only in severe cases, since methemoglobin normally reverses to hemoglobin at a constant rate. The general treatment for patients suffering from nitrobenzene poisoning is hospitalization and observation. Additional measures are not employed unless absolutely necessary. Treatment in cases of ingestion include stomach lavage and administration of a laxative followed by intravenous reducing drugs (e.g., methylene blue, ascorbic acid, Celthion) to minimize the methemoglobinemia. Oxygen treatments are used to restore the respiration to normal. Blood exchanges and transfusions are employed to dilute the hemolytic* products and provide hemoglobin; however, the effectiveness of these last measures is not completely accepted. The administration of alcohol, milk and oils, or any solvents of nitrobenzene is contraindicated.

It is extremely difficult to assess nitrobenzene toxicity to humans quantitatively, because very little data is available and responses vary so widely. Inhalation poses the least hazard, as the inhalation concentration is usually low enough to allow some elimination of the compound and to avoid build-up of lethal doses. However, cumulative effects may occur. The

*Broken blood cell.

toxicity of nitrobenzene vapor to man was reported by Von Oettinger (5), and is presented in Table IV.

The nitrobenzene threshold concentrations indicated below were determined by Andreeshcheva (49):

Odor detection	0.0182 mg/m^3
Eye sensitivity	0.0157 mg/m^3
Electroencephalograph disturbance	0.0129 mg/m^3

At present, OSHA regulations limit ambient nitrobenzene exposure to 1 ppm or 5 mg/m^3 (time weighted average over an eight-hour day) determined by the 1966 American Conference of Governmental Industrial Hygienists. This is also the threshold limit value (TLV) accepted in Germany and Sweden. The USSR has a limit of 3 mg/m^3 (41). Other recommended maximum concentrations have ranged from 5 ppm (5) to 0.008 mg/m^3. The latter is based on 73 days exposure of rats to this level with no effect on motor chronaxy,* cholinesterase** activity, or concentration of hemoglobin, methemoglobin or sulfhemoglobin (37).

Exposure by ingestion offers the opportunity for the largest acute doses of nitrobenzene, but this rarely happens accidentally. The Food and Drug Administration (FDA) has discounted approval of

*Time required for nerve excitation.

**An enzyme associated with nerve pulse transfer.

TABLE IV

EFFECTIVE LEVELS OF NITROBENZENE VAPOR ON HUMANS

Symptoms	Concentration in Air	
	mg/liter	ppm (volume)
1/2 - 1 hour without immediate or late effects	1 - 1.5	200 - 300
6 hours without material symptoms	0.3 - 0.5	60 - 100
several hours with slight symptoms	0.2 - 0.4	40 - 80
1 hour maximum inhalation without serious disturbances	1.0	200

Source: Von Oettinger, W.F. "The Aromatic Amino and Nitro Compounds, Their Toxicity and Potential Dangers. A Review of the Literature." Public Health Bulletin 271. U.S. Public Health Service, Washington, D.C., 1941.

nitrobenzene as an additive to food, therefore, its use as a food flavor substance has been curtailed.

Cutaneous absorption of the liquid offers the greatest potential hazard. Skin contact affords the opportunity for relatively fast uptake of large quantities, without sufficient time for metabolism, elimination, or methemoglobin reversal to occur. Thus, an accumulation of the compound with subsequent toxic effects is likely. There are no federal regulations concerning the use of nitrobenzene in dyes, so cutaneous absorption from dyed leather shoes and gloves is possible. Additionally, accidental spills and splashings of dyes on skin or clothing can provide toxic doses.

The literature indicates variability in toxic oral doses of nitrobenzene. In humans, lethal doses of nitrobenzene may vary by almost two orders of magnitude. For example, fatalities were reported from oral ingestion of 4 ml of nitrobenzene (in 10 to 22 hours) while recovery occurred after as much as 333 ml (5). Reports of both recovery and death from doses intermediate to the above are given, but data on mediating conditions (such as alcoholic intake or a full stomach) or purity of the compound were not presented. Children are more sensitive than adults, as evidenced by the severe effects caused by cutaneous absorption of dye from a diaper stamp (5). This may possibly be due to the lower blood volume of children. Table V lists dosages and toxic effects produced by nitrobenzene in man and other organisms.

TABLE V
TOXICOLOGICAL EFFECTS

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Human	inhalation	-	8 hours/day for 17 months factory worker (paint firm)	cyanosis, headache, fatigue, methemoglobinemia	22
	inhalation	- (poor ventilation)	8 hours/day for 1.5 months factory worker (paint firm)	cyanosis, headache, fatigue, methemoglobinemia, liver damage, hypotension	22
			8 hours/day for 4.5 months	above plus: liver and spleen enlarged and tender, hyperalgesia in extremeties	22
	inhalation	0.2-0.5 mg/l (40-100 ppm)	ca. 6 hours	slight effects, e.g., headache, fatigue	5
	inhalation	0.129 mg/m^3	-	threshold level for electroencephalograph disturbance	40
	inhalation	"large" amounts (poor ventilation)		hospitalized: day 1 - fatigue, headache, asthma 2 - vertigo, coma, cyanosis 3 - labored breathing, urine with almond odor, methemoglobinemia 7 - symptoms gone recovery after one month	31
	inhalation	acute	-	burning throat, nausea, vomiting, gastrointestinal disturbances, cold skin, livid face, cyanosis	5

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Human	inhalation	-	- nitrobenzene factory worker	intermittent symptoms: cyanosis, pallor and jaundice, pharyngeal congestion, headache, changes in blood cell composition (increased polymuclears and eosinophils)	5
	inhalation	6-30 µg/l	6 hours	retained 80 percent of vapor in lungs urinary excretion of p-nitrophenol (maximum in 2 hours, still detected after 100 hours)	23
	inhalation	-	- factory worker (rubber accelerator)	pregnant women: thickening of tissue in blood vessels, decreased placental absorption, necrosis in placental tissue	34
	inhalation	-	- factory worker (glass, porcelain)	changes in bone marrow, increased lymphoid cell production, impairment of copper metabolism and certain iron-containing enzymes	28
	inhalation	-	- industrial exposure	disturbance of motor impulses	30
	inhalation	acute	- factory worker (filled containers with nitrobenzene)	14 days: cyanosis, headache, backache, stomachache, vomiting ca. 21 days: drank beer and fell unconscious, cyanosis, dilated pupils, retarded respiration, weak pulse 1 year: intelligence dimmed 2 years: emaciated, atrophied muscles 3 years: memory failed 6 years: loss of perception of time and space (Korsakoff's syndrome)	6

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Human	cutaneous absorption	dye used in diaper stamps	-	babies: cyanosis, rapid pulse, shallow respiration, vomiting, convulsions, recovery in 24 hours	5
	cutaneous absorption	shoe dye	ca. 7 hours	unconsciousness after consumption of alcoholic beverages, death	6
	cutaneous absorption	0.5% by weight in paper	- (handled carbon paper)	dermatitis	35
	oral	-	from human milk	nurselings became cyanotic, recovery in 24 hours (mothers ate almond cake artificially flavored with nitrobenzene.	24
	oral	333 ml	one dose	maximum dose with recovery reported (following severe symptoms).	5
	oral	0.4 ml	one dose	minimum lethal dose reported	6
Rabbit	subcutaneous injection	0.8 mg/kg	daily	maximum dose not causing death	42
	subcutaneous injection	10-14 mg/kg	one	maximum effective dose: slow and lasting methemoglobinemia	5
	cutaneous absorption	0.7 gm/kg	-	52 hours: lethal	5
	intraperitoneal injection	0.5 gm/kg	one	reduced blood pressure and myocardial glycogen level	46

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Rabbit	intravenous	0.1 gm	daily or every 5 days	simultaneous doses of 2-20 ml ethanol increased severity of poisoning	57
	oral	9 gm	4 doses, one every 15 minutes	convulsions, death	5,6
	oral	4.8 gm	one	lethal instantly	5,6
	oral	700 mg/kg	one	lethal dose	3
	oral	600 mg	one	dizziness, loss of reflexes, methemoglobinemia, congestion of brain tissue. 12 hours - death	6
	oral	300 mg	one	fatigue for one week	43
	oral	50 mg/kg	one	tissue degeneration, especially heart, liver, kidney	67
	oral	>1 mg/kg	one	lowered hemoglobin, erythrocytes and lymphocytes; increased leucocytes	68
	oral	0.1 mg/kg	one	threshold toxic dose	68
Guinea pig	inhalation	saturated air (0.04 volume %)	2-5 hours	death following tremors, paralysis of hind legs	6
	inhalation		2-3 hours	death	6
	subcutaneous injection	0.2 gm/kg	every other day for 6 months	hemolytic anemia, loss of weight, decreased motor activity, fluxes in urinary excretion of 17-oxo-corticosteroids	44

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Guinea pig	oral	ca. 3 gm	one	0.5 hours: tremors, faint heartbeats, laborered respiration 2 hours: death	6
	oral	ca. 1.2 gm	one	immediately motionless, then complete recovery	6
	oral	50 mg/kg	<1 year	tissue degeneration, especially heart, liver, kidney	68
	oral	>1 mg/kg	one	lowered hemoglobin, erythrocytes, lymphocytes; increased leucocytes	68
	oral	0.1 mg/kg	one	threshold toxic dose	68
Rat	inhalation	5 mg/m^3	8 hours	metabolites excreted in 3 days	22
	inhalation	ca. 0.03 mg/m^3	daily, up to 98 days	increased ability to form sulfhemoglobin in preference to methemoglobin	48
	inhalation	0.06-0.1 mg/m^3	70-82 days	cerebellar disturbances, inflamed internal organs	69
	inhalation	0.008 mg/m^3	73 days	no effect	40
	oral	0.6 gm/kg	one	LD_{50}	56
	intraperitoneal injection	0.8 gm/kg	one	lethal	70

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Rat	subcutaneous injection	640 mg/kg	one	blood catalase activity decreased continuously over 96 hours	51
	subcutaneous injection	300 mg/kg	one	LD (14 days) - methemoglobinemia, anemia, sulfhemoglobinemia	18
	subcutaneous injection	200 mg/kg or 100 mg/kg	one daily for 10 days	methemoglobinemia, sulfhemoglobinemia, anemia	47
	subcutaneous injection	125 mg/kg	one	delayed embryogenesis, abnormal fetal development and embryo death, changes in polysaccharide composition of placenta	53, 54, 55
	subcutaneous injection	100-200 mg/kg	one	sulfhemoglobin (most regular and persistent form of hemoglobin) nitroxyhemoglobin, increased methemoglobin	49
Mouse	cutaneous absorption	480 gm/kg	-	30 min. - prostrate, motionless 24 hours - death	5
	intraperitoneal injection	1.23 gm/kg	one	40 min. - 67% dead	50
	intraperitoneal	1 gm/kg	one	10-15 min. - incoordination, comatose, shallow respiration several hours - regained coordination immediately before death - lost coordination again, respiratory arrest 48 hours - death	50

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Mouse	intraperitoneal injection	20 mg/kg	one	lethal dose	18
	intraperi-	12.3 mg/kg	one	10 minutes: 4.2% methemoglobin formed	50
	injection				
Cat	inhalation	saturated air (volume %: 0.04)	2-5 hours	death following tremors, paralysis of hind legs	6
	inhalation	-	2-3 hours	death	
	oral	2.4 gm	one	death in 12 to 24 hours	5,6
Dog	inhalation	"thick vapor"	1 1/2 hours	complete anesthesia and sleep	6
	intravenous injection	0.15-0.25 gm/kg	one	minimum lethal dose. lowered blood pressure, pulse rate increased then decreased, respiration stimulated until paralyzed	5
	oral	28.8 gm plus 6 gm	2 doses, 0.5 hours apart	immediate - agitation, then motionless 1 hour - convulsions, then motionless 4.5 hours - tremors, hind legs paralyzed 18 hours - death	6
	oral	24 gm	one	few hours - "stupid" 12 hours - deep coma, slow respiration, lowered skin temperature, stomach strongly alkaline	6

TABLE V (CONTINUED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Dog	oral	2.4 gm	one	1 hour - vomiting, then sleepy, continuing for 6 hours 6 hours - appeared normal 15-81 hours - rigid muscles 104 hours - death	6
	oral	0.75-1.0 gm/kg	one	minimum lethal dose	5
	oral	0.5-0.7 gm/kg	one	salivation, unrest, dizziness, tremors, increased pulse rate, sometimes convulsions	6
	oral	-	daily	formed methemoglobin continuously at "certain" concentration	71
Chicken	oral	1.2 gm	one	unsteady gait, recovery	6
	oral	2.4 gm	one	immediately unconscious 12 hours - death	6
Pigeon	inhalation	-	1 hour 2-3 hours	no effects death	6
Frog	inhalation	saturated air (volume %: 0.04)	-	general depression	5,6
	oral, subcutaneous injection or inhalation	-	-	paralysis of all movement, abolition of all reflexes, death	6

TABLE V (CONCLUDED)

Organism	Dose			Response	Reference
	Route	Concentration	Exposure Time		
Insects	inhalation	saturated air (volume %: 0.04%)	-	general depression, occasional leg tremors	6
Yeast spores (Actinomyces sphaeroides)	culture solution	0.001-0.004 Molar	2 hours	6-80% decrease in viability, more than 13% morphological changes	64
Micro-organisms	ambient water	630 gm/liter	-	inhibited 50% oxygen utilization	18
	in sludge	-	-	toxic to benzene-activated sludge	61
	in sewage	2-20 ppm	-	delayed or inhibited sulfate reduction by bacteria	62

2. Animals

Early experiments with nitrobenzene on animals established the following conclusions:

- o nitrobenzene exhibits toxic properties whether ingested, absorbed cutaneously, inhaled or injected subcutaneously
- o the size of the dose required to produce toxic effects is highly variable
- o toxic symptoms are inconsistent
- o a latent period often occurs between administration of nitrobenzene and the onset of symptoms
- o nitrobenzene need not be converted in the body into other substances before it can exert a toxic action
- o nitrobenzene forms methemoglobin in the blood.

In addition, some specific points are worth mentioning as they relate to later findings.

Metabolism. Wistar rats (22), exposed to 25 ppm nitrobenzene for eight hours, were found to excrete p-nitro- and p-aminophenol in ratios comparable to those in a woman exposed to industrial nitrobenzene vapors. The rat urine was analyzed over 24-hour periods for metabolic products. The times and rates of excretion are presented in Table VI.

A pattern of metabolic compound formation similar to that in rats and humans was found in rabbits. However, nitrophenol and aminophenol positions were reversed with the latter as the major excretory product. Rabbits were studied biochemically and pathologically to determine their processes of nitrobenzene detoxification (42). The maximum daily chronic dose (0.7 ml/kg) of nitrobenzene was

TABLE VI

NITROBENZENE METABOLITE EXCRETION IN RAT URINE

Hours After Ingestion	p-aminophenol	p-nitrophenol
	mμ moles/24 hrs/rat	
0	0	0
24	1,200	1,750
48	500	400
72	0	0

Source: Ikeda, M. and A. Kita. "Excretion of p-nitrophenol and p-aminophenol in the urine of a patient exposed to nitrobenzene." British Journal of Industrial Medicine 21(3):210-213, 1964.

injected subcutaneously. Metabolic products suggest the initial modes of detoxification to be amination and acetylation as well as hydroxylation to a lesser degree. The relationship is reversed in the second stage of detoxification and declines markedly in the terminal stage. The material was identified as p-aminophenol.

Urinary excretion of radioactive (14-C) carbon labeled nitrobenzene (0.25 ml) was almost complete within five days after intubation in rabbits (43). The major urinary metabolite was p-aminophenol (31 percent of dose), with p-nitrophenol (9 percent) and o-aminophenol (3 percent) in lesser amounts (see Table VII). In two days, 54 percent of the dose was absorbed by the tissues. Most nitrobenzene accumulated in the fat and intestinal tract tissues unchanged. By eight days, 8 percent of the material was located in predominately fat tissue, and no free nitrobenzene or odor was detected in the tissues, suggesting that only metabolites remained. Approximately one percent of the dose was expired as carbon dioxide and 0.6 percent as unchanged nitrobenzene in 30 hours. Excretion of 70 percent of the material through exhalation, urine and feces took place in 4 to 5 days. The remainder was slowly eliminated in urine and exhalation. No ring denitration products (e.g., phenol), nitrosobenzene, or azoxybenzene were detected. In one experimental guinea pig, Parke (43) found similar results, with the exception of no o-nitrophenol in the urine and decreased m-nitrophenol, perhaps as a result of reduction to aminophenols.

TABLE VII

FATE OF A SINGLE ORAL DOSE OF 14-C NITROBENZENE DURING 4-5 DAYS AFTER DOSING (0.25 ml)

Metabolite	Percentage of Dose (Average)		
Respiratory CO_2	1.0	2.0 in expired air	60.0 total
Nitrobenzene	0.6*		
Aniline	0.4**		
o-nitrophenol	0.1	58.0 in urine, all aromatic compounds	
m-nitrophenol	9.0		
p-nitrophenol	9.0		
o-aminophenol	3.0		
m-aminophenol	4.0		
p-aminophenol	31.0		
4-nitrocatechol	0.7		
Nitroquinol	0.1		
p-nitrophenylmercapturic acid	0.3		
(total urinary radioactivity)	(58)		
metabolized nitrobenzene in feces	9.0***		
metabolized nitrobenzene in tissues	15-20.0		
total accounted for	85-90%		

Source: Parke, D. V. "Studies in Detoxification. 68. The metabolism of [14-C] nitrobenzene in the rabbit and guinea pig." The Biochemical Journal 62(2): 339-346, 1956.

*0.5% in expired air; <0.1% in urine
**0.3% in urine; <0.1% in expired air
***6% dose present in feces as p-aminophenol

Physiological Reactions. Typical symptoms of nitrobenzene poisoning (anemia, fatigue) were observed in guinea pigs (0.2 g/kg subcutaneous injections every other day) with fluctuating urinary secretion of 17-oxo corticosteriods (44). Administration of ACTH (adrenocorticotropic hormone) and hydrocortisone had no effect on these symptoms but improved animal survival. Centrilobular necrosis of rat liver was observed as a result of nitrobenzene poisoning, but no glucose-6 phosphatase suppression or calcium influx occurred (45). The latter reactions are common in detoxification of hepatic poison. These observations support theories that nitrobenzene effects are a direct result of both blood changes and its accumulation in the cerebellar cells which affect the central nervous system.

Effects of acute and chronic doses of nitrobenzene on the blood of rats and rabbits included hypotension, reduced myocardial glycogen,* irregular heart beat, increased weight coefficient of the heart, and hemoglobin changes (46, 47, 48, 49). Sulfhemoglobinemia was considered more characteristic of poisoning and more persistent than methemoglobinemia. Nitroxyhemoglobin was also present in the blood (49). Kusumoto and Nakajima (27) found that nitrobenzene may act directly on hemoglobin without a chemical change. However, a much weaker methemoglobin formation occurs than with aminophenol, because nitrobenzene is not oxidizable and less structural change occurs in the hemoglobin (27).

*Heart muscle sugar.

Mice exposed to nitrobenzene showed greater resistance to methemoglobin formation than rats, dogs and humans. This is probably due to a higher rate of methemoglobin reductase* activity and not a more resistant hemoglobin (50). Blood catalase activity** in rats decreased significantly and continuously after a subcutaneous injection of 0.64 g/kg (51). Symptoms from lethal nitrobenzene doses in mice (8.1 m moles/kg, intraperitoneal) suggest central nervous system involvement, and include incoordination, coma and shallow respiration. Chronic respiratory administration to mice in small doses (dose not given) produced weight loss, anemia, respiratory insufficiency and significant changes in oxygen consumption and cerebral enzyme activity (52). Inhalation of nitrobenzene by mice (50 to 80 mg/liter) produced a pronounced congestive effect on lungs, heart, and kidneys after chronic and acute exposures. In a hundred-fold dose range of nitrobenzene (0.1 to 10.0 m moles/kg), no graded response was observed in methemoglobin levels in mice assayed after intraperitoneal injections (50). Table VIII presents data indicating the lack of correlation between methemoglobin formation and nitrobenzene dose.

Nitrobenzene was shown to be the active group in sensitizing mouse tumor cells to radiation (37). It was suggested that the effect was due to radical oxidation and increased cellular damage. Nitrobenzene compounds diffused into the tumors after intravenous injections to give levels equivalent to those in blood. A three-molar

*Enzyme reversing methemoglobin to hemoglobin.

**Enzyme associated with oxidation reactions.

Table VIII

PERCENT CIRCULATING METHEMOGLOBIN IN MICE
AFTER INTRAPERITONEAL INJECTION OF NITROBENZENE*

Dose (millimoles/kg)	Time After Exposure (Minutes)				
	0	10	20	40	60
0.1	0	4.2	3.9	2.0	0.4
0.5	0	3.9	4.1	3.6	2.8
1.0	0	3.0	1.5	1.6	3.3 ±1.9
5.0	0	8.3	9.8	10.2	9.0
10.0	0	5.5	7.2	2/3 dead	

Source: Smith, R. P., A. A. Alkaitis, and P. R. Shafer. "Chemically induced methemoglobinemias in the mouse." Biochemical Pharmacology 16(2):317-328, 1967.

*average of three animals

solution of nitrobenzene was found to reduce net cell proliferation 50 percent in cultures of hamster cells (38).

Nitrobenzene administered to pregnant rats caused dead and deformed embyos and changes in respiratory tissues (53, 54). Delay of embryogenesis and the appearance of abnormalities in fetuses resulted from injections of 125 mg/kg subcutaneously during the preimplantation and placentation periods, respectively. Normal placentation appeared to be upset because of changes in the redox processes in the placenta (55). Glycogen content was increased and polysaccharide composition changed in embryonic and placental tissue of rats injected with nitrobenzene during the period of placentation (53).

Lethal and acute effects of nitrobenzene were determined in fish (18). Toxic concentrations and the fish species studies were not identified. However, incomplete intravascular blood coagulation after death was reported.

Synergistic Effects. Possible synergistic action between nitrobenzene and 27 other industrial chemicals was tested in all possible pairs by intubation of rats (56). Mixtures of nitrobenzene and a particular chemical were tested. Compositions were prepared on the basis of the individual LD_{50} values of the pure components (56). Predicted and actual LD_{50} results did not vary significantly. However, intravenous doses of 0.1 ml nitrobenzene in rabbits were increased in severity by simultaneous doses of 2 to 20 ml ethanol. This agrees with

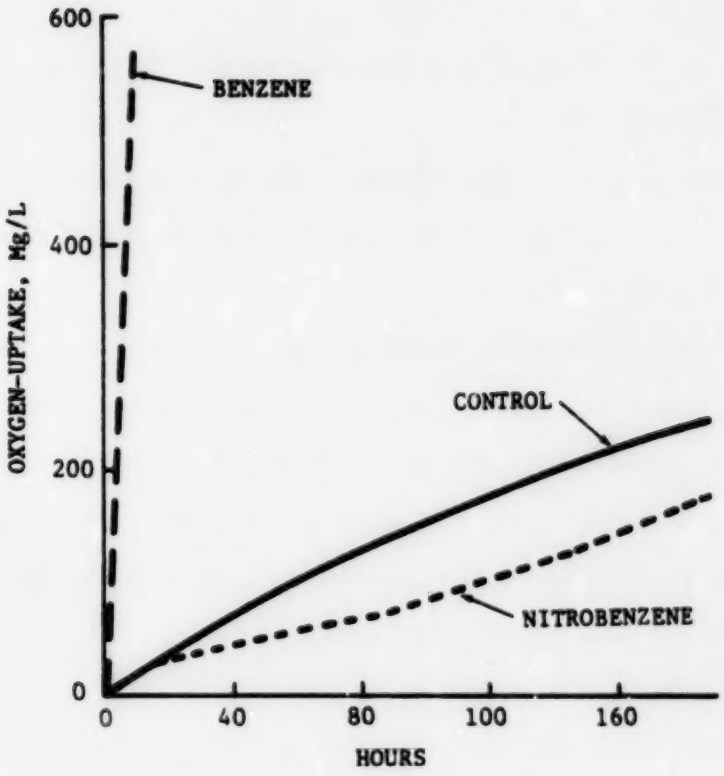
the clinical data discussed previously on the enhancing properties of alcohol on nitrobenzene poisoning in humans (57).

3. Bacteria

Studies using synthetically prepared sewage effluent showed complete biological degradation of nitrobenzene from solution (58 (58). Bacterial degradation of nitrobenzene was also observed in a model waste water system and in laboratory cultures (59). Nitrobenzene was almost completely removed in the aeration stage of a 2-stage waste water purifier. When Azobacter agilis was inoculated into the first stage aerator, it reduced the nitrobenzene concentration, but the reduction products could not be detected. Growing cultures, washed-cell suspensions and cell-free extracts of Nocardia erythropolis and Psuedomonas fluorescens reduced the nitro group of benzene to the amino level, probably via the nitroso and hydroxyamino stages (60). The nitroreductase is nonspecific and required reduced diphosphopyridine nucleotide as the hydrogen donor. Enzyme reactivity is stimulated by flavine adenine dinucleotide (FAD). It requires bivalent metal (e.g., MN^{++}, Mg^{++}, Fe^{++}), and is inhibited by hydroxylamine and nitrite.

Nitrobenzene was found to be toxic to benzene-acclimated sludge at 630 mg/liter, decreasing the oxygen uptake by 50 percent (see Figure 3) (61). Sulphate-reducing bacteria in sewage also had growth retarded by the addition of nitrobenzene (62). Hydrogen sulfide production was inhibited by 2 to 20 ppm addition of nitrobenzene. Allen (62) suggests that nitrobenzene may retard the lowering of the

FIGURE 3
OXIDATION OF NITROBENZENE BY BENZENE-ACCLIMATED ACTIVATED SLUDGE



Source: Malaney, C. W., and R. E. McKinney. "Oxidation abilities ties of benzene-acclimated activated sludge." Water and Sewage Works (August):302-309, 1966.

oxidation-reduction potential of the sewage to the region favorable for sulfate-reducing anaerobe growth. Azobacter vinelandii did not utilize nitrobenzene, probably because it is not reduceable to ammonia (63)

4. Others

Spores of the yeast Actinomyces sphaeroides (Streptomyces spheroides) showed a decrease in viability of 6 to 80 percent after treatment for two hours with 0.001 to 0.004 molar nitrobenzene (64). More than 13 percent of the spores showed morphological changes at 0.004 molar levels.

In the early 1900's, nitrobenzene was recommended for use as a fumigant for the extermination of external parasites on domesticated animals (6). Hosts were killed in shorter periods of fumigation than were the parasites (fleas or biting lice). However, at low temperatures (<20°C) and for short periods (<1.5 hours), parasites may be stupified and collected without any damage to the host.

Two arachnids showed limited metabolism of 14-C nitrobenzene (65). In vivo reduction to aniline by the cattle tick (Boophilus microplus) and the tiger spider (Nephila plumipes) accounted for 3.5 percent and 5.4 percent respectively, of the recovered 14-C. Hydroxylation of aniline and nitrobenzene produced amino- and nitrophenols in the amounts of 8.3 percent and 6.5 percent by the tick and spider, respectively. Thus, aromatic hydroxylation of nitrobenzene was somewhat more important than nitroreduction in these arachnids.

Nitrobenzene was also reduced by tissues from the Madagascar cockroach, Gromphadorhina portentosa (66). The highest activity was found in the excretory tubules, followed by the midgut and fat bodies. Nitrobenzene reduction was inhibited by aerobic conditions and decreased by the omission of glucose-6-phosphatase and nicotinamide adenine dinucleotide phosphate (NADP). It was found that nitrobenzene could be reduced without enzymes by addition of reduced flavine monoculeotide (FMNH). The unreduced form (FMN) increases nitrobenzene reduction in cockroach tissues, apparently as a result of the enzyme-activated reduction of FMN, followed by the hydrogen transfer to nitrobenzene without enzyme mediation.

Further evidence of the oxidizing properties of nitrobenzene was shown by the quenching of chlorophyll-a fluorescence (67). Spinach chloroplasts and fragments were quenched in vivo by the addition of nitrobenzene.

C. AMBIENT CONCENTRATIONS, POPULATION AT RISK AND MEASUREMENT TECHNOLOGY

It has been estimated that 8.3 million pounds of nitrobenzene are lost during production each year (18). Most nitrobenzene lost will be in the acid wash effluent discharge produced during acid regeneration and the raffinate* water layer used to extract the acid from crude nitrobenzene prior to distillation. At one plant, the loss of nitrobenzene in the waste water totaled 0.09 percent (72) and at another, 2.0 percent (85). Other organic wastes which contain some nitrobenzene are incinerated. The remainder of the nitrobenzene losses will be atmospheric emissions.

Nitrobenzene may be vented to the atmosphere at the reactor and the acid condenser. Both vents are normally equipped with absorbers or condensers but some nitrobenzene and nitrogen oxides will escape (73). Industry does not monitor extra-plant nitrobenzene levels but assumes them to be very low, far below the inner plant levels (these in turn remain below the TLV) (85).

Instead of vent scrubbers, some manufacturers of nitrobenzene maintain material under a carbon dioxide blanket to prevent atmospheric emissions. The producers believe these measures are adequate to maintain a permissible concentration (<1.0 ppm) of nitrobenzene in work and storage areas. At a nitrobenzene factory in England, *in situ* studies showed that workers received a maximum nitrobenzene

*The liquid product resulting from the extraction of a liquid with a solvent.

dose of 70 mg/day by vapor absorption through skin and lungs (23). At a dye stuff factory, the maximum dosage was 80 mg/day, and most workers absorbed less than 35 mg/day (23). A "reasonably safe" nitrobenzene uptake was reported as 35 mg/day by Salmowa et al. (23).

Ambient levels of nitrobenzene may be estimated in the vicinity of a plant releasing nitrobenzene through ventilation and exhaust of internal air. Atmospheric dispersion from this plant would distribute the gaseous emissions downwind, as affected by the turbulent diffusion of the air. Hypothetical plant conditions can be used as inputs to a Gaussian plume equation* from Turner's Workbook of Atmospheric Dispersion Estimates (76). The basic diffusion equation should be modified, however, due to the effect of the plant itself on the flow of air. Mechanical turbulence in the wake of a building tends to produce

*Ground level downwind concentrations resulting from a point source are predicted by the following equation:

$$X(x,0,0;H) = \frac{Q}{\pi \sigma_y \sigma_z u} \exp\left\{-1/2 \left(\frac{H}{\sigma_z}\right)^2\right\}$$

where: Q = uniform emission rate (grams/sec)
u = mean wind speed affecting the plume (meters/sec)
H = effective stack height, that is sum of stack height plus plume rise (meters)
σ_y = horizontal dispersion coefficient evaluated in terms of downwind distance to the point for which the concentration
σ_z = vertical dispersion coefficient evaluated in terms of downwind distance to the point for which the concentration is being computed (meters)
x = ground level concentration along plume axis (grams/meter^3).

aerodynamic downwash, resulting in the fairly rapid diffusion of a gaseous emission down to the ground. This region of disturbed flow extends downwind a distance equal to several times the height of the building. While this situation does not result in a Gaussian distributed plume, it is proposed that a modification of the usual formula still can be employed. This is because the turbulent mixing in the wake of the building is assumed to be distributed uniformly in the vertical direction, analogous to the situation where a plume is trapped below an inversion layer. A limited vertical mixing height can be estimated using the equation:

$$X(x,0,0;H) = \frac{Q}{\pi \sigma_y u\,(.8L)}$$

where L is the limit of the mixing depth. In the case of an isolated rectangular building it is assumed that L equals 1.5 times the building height.

In addition, there is a horizontal wind turbulence which is assumed to result in an initial horizontal plume spread equal to the width of the building normal to the wind direction. This is analogous to an area source emission where the area equals the building top. This can be modeled using a further modification of the basic Gaussian diffusion equation. A virtual point source is assumed upwind from the building at a distance where the plume spread (for the given stability conditons) would equal the crosswind width of the building.

The modified diffusion equation is therefore:

$$X(x,0,0;H) = \frac{Q}{\pi \sigma'_y u\ (1.2h)}$$

where h is the building height and σ'_y is based on the downwind distance to the receptor point plus the upwind distance to the virtual point source.

A downwind distance of 500 meters from the emission source has been chosen as the hypothetical measurement point. This value was chosen to represent a point within the perimeter of the largest production facility (manufacturing 310 million lbs/year). Ground level concentrations at this point were estimated as 1.56^3 mg/m .*

An individual positioned 500 meters downwind would have to remain for about 25 hours to inhale an amount equivalent to the exposure of a worker to 40 mg/m^3 during an 8-hour work day, the maximum dosage recommended by the American Conference of Governmental Hygienists (12). This calculation has not taken into account any control efforts to mitigate the effects of dispersion of nitrobenzene (calculated from the production loss of the entire industry). Most, if not all, plants will have employed some form of air emissions control technology to reduce the resulting nitrobenzene plant emissions to a permissible level. Individual industries refused to

*Additional assumptions: (1) nitrobenzene is nonreactive; (2) atmospheric stability class is neutral; (3) wind speed is 6 meters/sec.; (4) plant operates 24 hours a day, 330 days per year; and (5) the facility is 50 feet high (15.24 m) and 100 feet wide (30.48 m).

disclose their emissions data, so the above estimate may be viewed as an upper level. However, it emphasizes the need for effective control of air emission of nitrobenzene.

Threshold limit values (TLV) are set by the American Conference of Government Industrial Hygienists for nitrobenzene at 1 ppm or 5 mg/m^3 in air for an 8-hour working exposure (12, 41). A wide variation in individual susceptibility, discomfort or illness, may be experienced by different subjects at the TLV. A sweet nitrobenzene odor can be detected at 0.5 ppm and may prove a useful indicator of high exposure (7).

There is some evidence that atmospheric formation of nitrobenzene is possible by the photochemical reaction of benzene with nitrogen oxide. Toxicity symptoms of nitrobenzene exposure are similar to those of seriously stricken victims of Japanese photochemical smog (75).

Qualitative spot tests for nitrobenzene depend upon the characteristic color developed by its reaction with various reagents. If nitrobenzene is heated or fused with diphenylamine, a yellow to red complex is formed. Likewise, partial reduction to nitrosobenzene followed by treatment with Na_2 $(Fe(NH_3)(CN)_5)$ in alkaline solution gives a dark purple-violet compound. These tests lack specificity, however, in that other nitroaromatic compounds give colored compounds whose shades may differ only slightly from that of nitrobenzene (77). In the absence of nitrophenols and nitrotoluenes, colorimetric methods can be used with an accuracy of 0.1 to 0.2 percent.

Nitrobenzene may be detected in air by passing the sample through an activated charcoal filter (5). Adsorbed nitrobenzene is then reduced to aniline, distilled off with steam and determined in the distillate by diazotization and coupling with aryl salt. It is said that concentrations of 0.2 to 100 ppm may be determined in this way.

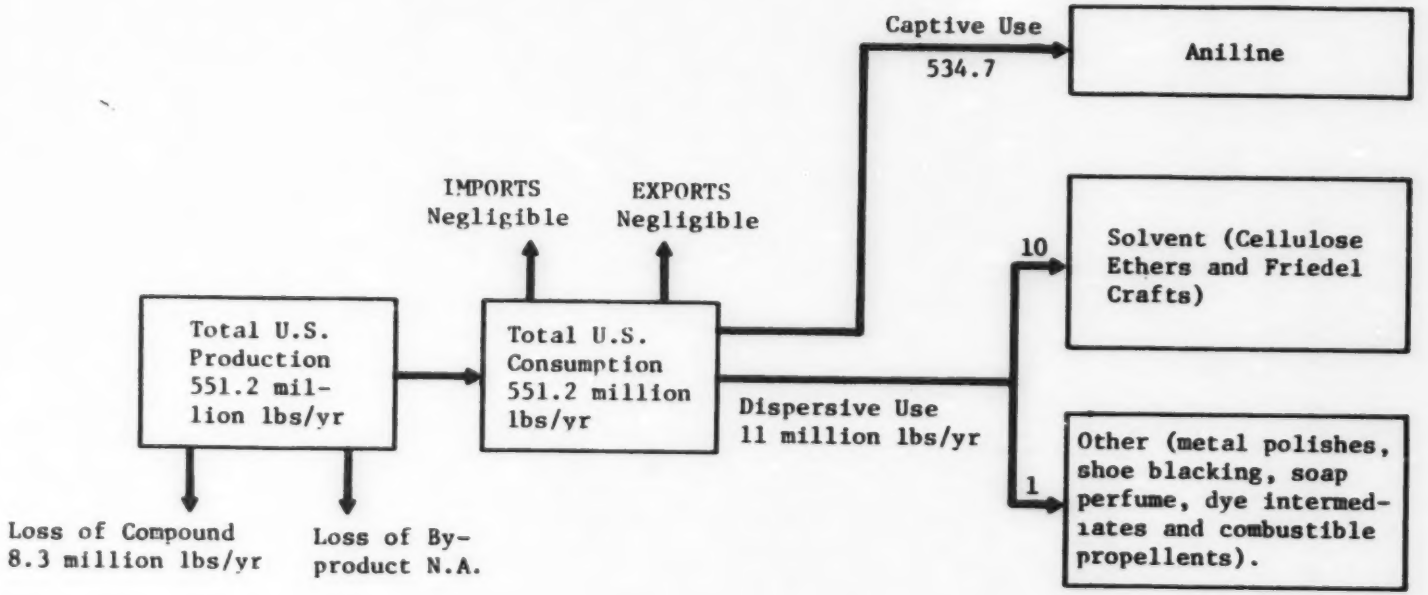
Biological monitoring of exposed workers is also possible since the p-nitrophenol excretion in the urine can be measured as an indicator of both skin and inhalation exposure (23). The urine level will reach a maximum level 2 hours after exposure and may be detected up to 100 hours later.

D. SOURCES

Domestic production of nitrobenzene was 690 million pounds per year in May of 1971 (18). During the last decade, an average annual production increase of 10 percent has been observed, which may ultimately decrease to 7 percent because of benzene shortages (78).

Commercial demand for nitrobenzene is outlined in Figure 4 and summarized in Table IX. The usual method for commercial manufacture of nitrobenzene is the direct nitration of benzene using a solution of sulfuric and nitric acids. The holding time required for the reaction cycle is two to four hours. It is carried out in a closed, jacketed system equipped with internal cooling coils (73). Reactors are cast iron or steel kettles designed for 1000- to 1500-pound charges of benzene and are equipped for efficient agitation. The acid used for nitration is composed of 53 percent sulfuric acid, 39 percent nitric acid, and 8 percent water. The sulfuric acid forms a series of hydrates with water; when its concentration is high, the nitric acid ionizes ($HNO_3 \rightarrow NO_2^+ + OH^-$), and nitration of benzene occurs in preference to oxidation. Sulfuric acid also permits mutual solubility of reagents (allowing increased reaction rates) and increases the heat capacity of the solution (79). The benzene requires no feed preparation and is charged to the reactor with some spent acid. The major acid mixture addition is slowly charged below the surface of the benzene. The temperature during charging is maintained at 55°C. This reactor tank vents to

FIGURE 4
SUMMARY OF PRODUCT RELEASE INFORMATION FOR NITROBENZENE



Source: Brown, S. L., F. Y. Chan, J. L. Jones, D. H. Liu, K. E. McCaleb, K. N. Sapios, and D. E. Schendel, "Research Program on Hazard Priority Ranking of Manufactured Chemicals" SRI Project ECU-3386. Stanford Research Institute, Menlo Park, Calif., 1975.

TABLE IX

COMMERCIAL USES OF NITROBENZENE

Use	Percent
Aniline	97%
Solvent (Cellulose ethers Friedel Crafts)	2%
Metal Polishes	
Shoe Blacking	
Soap Perfume	
Dye Intermediate	
Combustible Propellant	

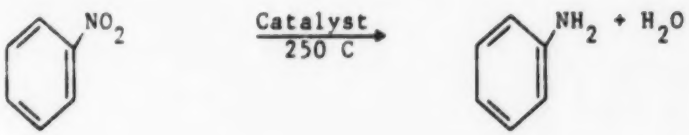
Source: S. L. Brown, F. Y. Chan, J. L. Jones, D. H. Liu, K. E. McCaleb, K. N. Sapios, and D. E. Schendel. "Research Program on Hazard Priority Ranking of Manufactured Chemicals" SRI Project ECU-3386. Stanford Research Institute, Menlo Park, Calif., 1975.

an absorber which is suspended above an emergency drown tank filled with water. Near the end of the reaction cycle, the temperature is raised to 90°C to drive off water and promote completion (73).

When the reaction is complete, the batch is transferred to a separator tank where the crude nitrobenzene separates from the spent acid by gravity. There is a 0.5 percent loss of yield of nitrobenzene in the spent acid.

The reaction dilutes the acid mixture, thus a sulfuric acid concentrator is an essential part of the nitration process. Acid regeneration represents a significant cost saving in the production process (1).

Nitrobenzene manufacture is closely tied to aniline production, because ninety-seven percent of all nitrobenzene is converted to aniline by vapor phase reduction (79).



This is a continuous process with nitrobenzene production as the first step. Thus, no storage is necessary and any possible harmful effects from storing of handling or crude nitrobenzene are eliminated. The demand for aniline has been increasing in proportion to its use in the manufacture of a number of products. The main products are:

thiazole vulcanization accelerators (rubber) (60 percent), diphenylamine (dyes) (15 percent), hydroquinone (photographic developing) (9 percent), medicinals (6 percent), resins (6 percent), varnish and shoe blacking (4 percent) (79).

Reduction of nitrobenzene to aniline is only one of several routes to produce aniline. However, it seems to occupy a stable position, and new technical developments have recently been applied to improve conversion efficiencies. Based on aniline's future, it seems likely that the future demand for nitrobenzene, as a precursor of aniline, will continue to increase.

There are seven companies in the United States that produce nitrobenzene at nine major facility locations. These locations are grouped into three areas: The Gulf Coast, the Pennsylvania (Philadelphia)-New Jersey area, and in West Virginia along the Pennsylvania-Ohio border as shown on Figure 5. These plants have a total capacity of 1065 million pounds per year and data by plant location are given in Table X.

Separate data are not reported by the Tariff Commission for aniline and nitrobenzene. Combined total imports of 1,170,007 pounds in 1970 represent only 0.25 percent of annual domestic production. Exports amounted to about 1 percent of the annual aniline production in 1968 (80).

Dispersive uses of nitrobenzene account for only 3 percent of total production, or about 20 million pounds per year (1971) (18).

FIGURE 5
GEOGRAPHIC DISTRIBUTION AND RELATIVE CAPACITIES OF NITROBENZENE PRODUCTION FACILITIES

TABLE X

U.S. NITROBENZENE PRODUCTION CAPACITY (1975)*

PRODUCER	LOCATION	CAPACITY[1] (millions of pounds)
Allied Chemical Corp.	Moundsville, West Virginia	55
American Cyanamid Co.	Bound Brook, New Jersey	85
	Willow Island, W. Virginia	60
E.I. duPont de Nemours & Co., Inc.	Beaumont, Texas	310
	Gibbstown, New Jersey	200
First Mississippi Corp.	Pascagoula, Mississippi	135
Mobay Cehmical Corp.	New Martinsville, West Virginia	135
Monsanto Co.	Sauget, Illinois	10
Rubicon Chemical Inc.	Geismar, Louisiana	75
TOTAL		1065

*Source: Stanford Research Institute, Directory of Chemical Producers, Menlo Park, Ca. 1975.

[1] As of 1 January 1975.

See Figure 5 and Table X. Of this, the bulk is used as a solvent in production of cellulose ethers and in Friedel-Crafts alkylations, while the remaining 1.0 percent is used in the manufacture of dye intermediates, metal polishes, shoe blacking, soap perfume and propellants (79).

If nitrobenzene is used to make aniline, crude nitrobenzene from the separator is transferred directly to the aniline process. For higher purities of nitrobenzene, the crude material is washed with water, dilute sodium carbonate, then water again to remove the spent acid. This product is distilled to obtain the pure nitrobenzene product known as oil of myrbane (82).

In contrast to the above-described batch process for nitrobenzene production, new plants use a continuous process, as typified by the Biazzi process (1). The sequence of operations is essentially the same as in the batch process, the main differences being the use of smaller reaction vessels, lower nitric acid concentrations, and higher reaction rates obtained by increased agitation due to smaller reaction volumes. Other new methods for nitrobenzene production involve continuous vapor phase nitration in tubular reactors. In an experimental tubular reactor, conversions as high as 99.3 percent were attained (1). This system may be considered in future plants because of its high efficiency, high yields, and lower capital and equipment costs.

Production losses are estimated to release 8.3 million pounds of nitrobenzene if sulfuric acid regeneration is practiced (82).

Principal production losses occur through the reactor and acid concentration vents, and in the spent acid (83).

E. CONTROL TECHNOLOGY

Nitrobenzene is classified by the Department of Transportation as a poisonous liquid, Class B (7). Handling of the material should be performed in well ventilated areas with workers adequately protected. When treating nitrobenzene spills or during cleaning of storage tanks, similar precautions should be taken. Small amounts may be disposed of by incineration in a deserted area. If permissible (in accordance with state and local laws), small amounts may be diluted with water to a less than one percent slurry and discharged into municipal sewers. Wastes containing nitrobenzene may also be buried in a land fill set aside for toxic wastes located away from public area and from which there is no leaching (7).

Nitrobenzene is highly toxic but may be handled safely if proper precautions are followed. It is an oily, fat soluble liquid and will absorb readily through the skin or by inhalation of vapor under conditions of inadequate ventilation. Protective clothing fabricated from butyl rubber is an effective deterrent and should be worn by plant workers. Emergency showers should be provided in areas of potential exposure so that immediate removal of contamination is possible. Contaminated clothing should be removed promptly and thoroughly laundered; contaminated shoes or gloves should be destroyed in a proper fashion or incinerated. Showers should be taken after each work exposure in cool water (not hot) with soap. Employees in hazardous areas should be checked daily since chronic methemoglobinemia

occurs initially without symptoms. All plant employees should have physical examinations every six months which include hemoglobin determination, blood counts and urinanalysis. Subjects with cardiovascular or renal disease of significant severity should not be employed as they are more sensitive to nitrobenzene's effects than the normal population (12).

The minimum personal protection equipment that should be available to employees are: safety goggles and face shields, self contained breathing apparatus or air line masks (for spills cleanup or tank cleaning), rubber safety shoes, and fresh daily clothing changes.

Tank cleaning and cleaning of spills demand special safety precautions. High exposure levels are quite possible, and persons involved in these operations must be closely monitored for toxic effects (7). Since nitrobenzene is 4.2 times heavier than air, design of vent locations should include several close to floor level.

Workers in industrial or commercial operations producing or using nitrobenzene naturally incur the highest exposure risk. It is essential that careful controls be implemented such that time weighted ambient concentrations do not exceed 1 ppm. Immediate cleanup of spills, periodic inspection of equipment, repair of damaged equipment and immediate repair of leaks must be strictly preformed to minimize atmospheric emissions and accidental skin contact. Waste materials from cleaning operations which are contaminated with nitrobenzene should be stored in a well ventilated area prior to disposal.

Several methods are available to control atmospheric emissions of nitrobenzene:

o Proper Design of Venting Appartus. Reactor vents go to an absorber packed with activated charcoal where nitrobenzene is incinerated in the presence of water to produce nitric acid. Unreacted air and a small amount of NO_x pass out the vent. The nitric acid so produced is combined with the spent acid from the reactor and sent to the acid regeneration facility. This procedure offers a small potential source of residual nitrobenzene but provides the economic advantages of nitric acid recovery.

o Proper Location of Vents. Within the plant it is important to maintain a high air flow and to have filtered vents at points near where spills or leaks are likely to occur. Since nitrobenzene is 4.2 times heavier than air, floor vents should be required in addition to wall and ceiling vents. A good air flow can greatly reduce the airborne nitrobenzene concentrations within the work area.

Most nitrobenzene residuals are released as effluent discharges in the waste wash waters.* Hence, water pollution by nitrobenzene is a most important item to control. The following control methods have been used effectively:

*Nitrobenzene, soluble in water to the extent of approximately 0.2 percent at 20°C, is odorous in water at low concentrations (0.03 mg/l has been reported as the threshold level). Acute aquatic toxicity occurs at about 30 mg/l and may inhibit biochemical oxidation of other substances in addition to resisting biodegradation.

o Incineration of Organic Wastes. The flaring of organic wastes is one of the main ways to eliminate liquid nitrobenzene or nitrobenzene contaminated materials.

o Recyclying Process Water Between the Absorber and the Stripper. Water is used in the absorber to react with NO_2 to produce nitric acid, the water from the stipper could be recycled for this purpose.

o Use of Waste Water in Cooling System. Some of the waste water from the washes can be cycled through the jacket of the reactor vessel to control the heat of reaction.

o Waste Water Used for Quencher. Since the reaction vessel in the batch production procedure is suspended above a quencher tank, the water used for this purpose need not be of high purity and waste water would be adequate.

o Physical Separation. The relative densities of nitrobenzene and water make physical separation possible. Extraction with solvents and steam stripping are also possible separation procedures which would permit recapture of nitrobenzene for return to the process stream (85).

o Acid Neutralization. It is likely that the waste water coming from the washing of nitrobenzene to produce oil of mirbane contains acid wastes which can be neutralized by passage through a limestone slurry bed or column.

o Dentrification. Excess nitrogen can also be removed from nitrified effluents using biological dentrification methods, ammonia

stripping or ion exchange resins (73). Azobacter agilis, Nocardia erythropolis and Pseudomonas fluoriscens have been shown to reduce the nitrobenzene level in sludge to a point where it cannot be detected (58, 59, 60).

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